ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Polymorphisms in FoxO 1a and 3a Gene Family and Its Relation to Obesity.

Safaa El- Karaksy[a], Shadia Hassan Ragab[b] , Noha Shaheen[a], Dina Farouk El Gayar[a], Dina Kandil[b]*, and Mahmoud Kadry El- Masry[b].

[a]Clinical Pathology Department, Cairo Univeriaty, Kasr El Anni Street, Giza, Egypt.
[b]Clinical Pathology Department, National Research Centre, El Bohoos street, Giza, Egypt .

ABSTRACT

Obesity, a major public health concern, is a multifactorial disease caused by both environmental and genetic factors. We hypothesized that superfamily FoxO genes, play role in the possible etiology of obesity as well as their correlation with increased risk of its complications, that's to say its correlation to increased risk of diabetes and dyslipidemia with consequent cardiovascular complications. The present study was designed to evaluate the possible association between FoxO genes especially 1a & 3a polymorphism and obesity as well as increased risk of its complications. This case control study was conducted on fifty obese patients with BMI > 30 kg/m² and fifty control healthy subjects. All enrolled cases and controls were subjected to genotyping for FoxO1a and FoxO 3a by real timing PCR as well as laboratory investigations including lipid profile, fasting and postprandial blood sugar, fasting insulin and insulin resistance. Obese patients had significantly higher incidence of FoxO1a polymorphism when compared to control group (P=0.04) while regarding FoxO3a no such significant relation could be detected (P= 0.2). dyslipidemia was manifested in the form of elevated cholesterol, triglyceride and low-density lipoprotein (LDL) levels in the obese patients compared to the healthy controls. our results showed that BMI was positively correlated to cholesterol, triglyceride and LDL levels in addition obese patients had insulin resistance with high fasting insulin levels. In addition, comparing patients with wild FoxO1a and FoxO3a genes and non wild form regarding the laboratory data (lipid profile, FBS, PPBS, fasting insulin and insulin resistance) no statistical significance between these two groups and their laboratory data except for fasting insulin (P=0.01) and insulin resistance(P=0.01) with FoxO1a and HDL (P=0.02) and fasting insulin (P=0.01) with FoxO 3a. FoxO1a gene polymorphism is associated with increased risk of obesity will FoxO3a polymorphism is not.

Keywords: obesity, FoxO1a, FoxO3a, gene, polymorphism

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Obesity is a complex multifactorial and heterogeneous condition with an important genetic component. It is defined as excess body fat by positive energy balance. It is associated with metabolic disease such as type 2 diabetes, hypertension, and coronary heart disease. Obesity also increases the risk of several malignancies in breast, colon, pancreas, and endometrium [1].

Obesity is a major health priority in the United States, as well as globally and associated with multiple co morbidities and reduced life expectancy. Effective management of obesity involves producing an intervention plan tailored to the individual patient. Potential contributory factors to weight gain, including dietary habits, physical inactivity, associated medical conditions, and medications, should be identified and addressed. Lifestyle interventions comprising diet modification, physical activity, and behavior therapy are fundamental to the management of obesity [2].

The worldwide prevalence of obesity more than doubled between 1980 and 2014. About 13% of the world's adult population [11% of men and 15% of women] were obese in 2014 .

The most commonly used method used today for classification an individual as overweight or obese is based on BMI [Body Mass Index] a value that is determined by dividing body weight in kilograms by square of height in meters. In adults, overweight is defined by a BMI >25 kg/m2, and obesity is defined by a BMI >30 kg/m2 regardless the sex [3].

Obesity is heritable and predisposes to many diseases . The prevalence of maternal obesity is increasing at an alarming rate. Even more disturbing is that maternal obesity increases susceptibility of offspring to developing metabolic disease later in life and therefore contributes to a vicious cycle of transgenerational transmission of disease [4].

Chronic diseases are substantially increased in association with obesity as declared by 10 times increase in diabetes,3-10 times increase in osteoarthritis,2-3 times increase in cardiovascular disease and 50% increase in cancer deaths [5]. The intraabdominal visceral deposition of adipose tissue, which characterizes upper body central obesity [assessed by waist circumference and/or waist: hip ratio] is a major contributor to the development of hypertension, elevated plasma insulin concentration and insulin resistance, hyperglycemia and hyperlipidemia [metabolic syndrome] [6].

Recently, major advances in obesity research emerged concerning the molecular mechanisms contributing to the obese condition [2].

Forkhead box [FoxO] proteins are a large family of factors that share a highly homologous Forkhead DNA-binding domain but diverge in the remaining sequences. These factors have been implicated in differentiation and developmental processes in both mice and humans. In particular, the three members of the FoxOa subfamily, FoxOa1, 2 and 3, which are most closely related to the archetype Drosophila FORK HEAD protein, have been shown to transcriptionally control early development, organogenesis and metabolism in mice. Through a genetic screen to assess the role of each Forkhead factor family member in adipocyte differentiation, we demonstrated that FoxO3a is the only Forkhead protein positively affecting adipocyte differentiation [7].

The O subfamily of forkhead box [Fox] transcription factors are direct targets of insulin action, regulating cellular metabolism and survival in response to nutrient and environmental stress. In addition, FoxO proteins appear to play pivotal roles in the transcriptional cascades that control differentiation in preadipocytes, myoblasts, and endothelial cells [8].

FoxO1 represents the predominant FoxO isoform present in adipose tissue. It represents an important mechanism regulating energy homeostasis and insulin sensitivity in obesity [9].

Recent research has demonstrated that FoxO1 negatively regulates adipogenesis by binding to the promoter sites of PPARG gene and preventing its transcription. Rising levels of PPARG are required to initiate adipogenesis; by preventing its transcription, FoxO1 is preventing the onset of adipogenesis [10].

On the other hand, the FoxO Transcription factors are direct targets of insulin action, regulating cellular metabolism and survival in response to nutrient and environmental stress. It is found that FoxO1a promotes the transcription of genes that increase glucose production. Moreover, because diabetes increases oxidative stress through the generation of reactive oxygen species it is also possible that an increase in FoxO-dependent transcription mediates the deleterious effects of hyperglycemia (so-called "glucose toxicity") [11].

FoxOs are also involved in lipid metabolism through the regulation of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase and sterol carrier protein gene expression [12]. The mRNA levels of three FoxO isoforms were altered in response to fasting and refeeding, which suggests that the genes respond differently to nutritional and hormonal factors [13].

Thus, the aim of the present research was to study the relation between FoxO genes especially 1a & 3a and obesity aiming at developing novel approaches for the prevention and treatment of obesity.

MATERIAL AND METHODS

Hundred blood samples from both sexes had been taken from fifty obese adults and fifty normal healthy volunteers ranging in age from 20 to 60 years old. The fifty healthy volunteers had been served as the control group with BMI less than 25 kg/m² . The fifty obese adults were classified according to their BMI into overweight adults with BMI > 25 kg/m² and obese with BMI > 30 kg/m². They were recruited from outpatient clinics at the National Research Center of Egypt (NRC) and Kasr El Ainy University Hospital. The protocol of the study was read and approved by the Ethnic Committee of National Research Centre and Kasr El Ainy University Hospital.

Exclusion criteria

Patients with medical conditions as hypothyroidism, Cushing syndrome, diabetes mellitus and obesity with mental retardation e.g. pader willi syndrome, Laurence-Moon-Bieddl and Cohen Syndrome were excluded from our study.

All subjects in the study were subjected to medical history taking, through clinical examination with emphasis on any complications or medications, blood pressure measurement (according to American Heart Association guidelines), anthropometeric indices[Measurement of body weight to the nearest 0.1 kg with balance scale, measurement of height to the nearest 0.1 cm and calculation of BMI using the standard equation (BMI= weight (Kg)/ height ² (m²)] . Determination of serum Lipid profile, fasting and post prandial blood glucose levels, fasting insulin for insulin resistance using (Homa-IR) were done. Genetic testing for FoxO gene 1a& 3a polymorphism was performed using Real Time PCR.

For each patient and control, venous blood was drawn after overnight fasting and two hours post prandial.

Determination of lipid profile and glucose was performed by (Olympas AU 400 : Olympus diagnostic, Japan). Estimation of fasting Insulin levels was performed by chemiluminescene assay using (Immulite1000: Seimens).

Detection of polymorphism in FoxO gene 1a& 3a

Genomic DNA was extracted from lymphocytes using the FavourPrep Blood Geonmic DNA Extraction Mini kit Cat.No.: FABGK 001-1.

RT- PCR assay was performed using the LightCycler system (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions, PCR amplification was performed by using the primer pairs, for Foxo 1a: forward primer 5'CTg gCT CTC ACA gCA ATg AT 3' ,reverse primer 3'CAC CAT AgA ATg CAC ATC CC5', and for Foxo 3a forward primer 5'ggg gAg TTT ggT CAA TCA gA3', reverse primer 3'TTT gCA TAg ACT ggc TgA Cg5'.

ISSN: 0975-8585

Statistical analysis

Statistical package for social science (SPSS, Chicago, Illinols, USA) program version 21was used for analysis of data.

RESULTS

This case control study included fifty obese patients with BMI > 30 kg/m² {30 males (60%) and 20 females (40%) their ages ranged from 35 to 56 years with mean value 45.6± SD 13.9, their height ranged from 162.3 to 173.0 cm with mean value 166.8± SD 6.2, their weight ranged from 96.8 to 110 kg with mean value 105± SD 13.7, their BMI ranged from 34.5 to 41.8 kg/m² with mean value 37.9± SD 5.6} and fifty control group BMI < 25 kg/m² {28 males (56%) and 22 females (44%) their ages ranged from 25 to 55 years with mean value 40.4± SD 15.2, their height ranged from 162.3 to 170.8 cm with mean value 166.9± SD 6.1, their weight ranged from 59.0 to 66.0 kg with mean value 63± SD 6.3, their BMI ranged from 21.6 to 23.2 kg/m² with mean value 22.5± SD 1.5}. Regarding the laboratory data of patients, their total cholesterol ranged from 155.5 to 241.0 mg/dl (mean 206± SD 68.1), their TG ranged from 90.3 to 154.3 mg/dl (mean 121.2± SD 40.7), their HDL ranged from 42.0 to 61.5 mg/dl (mean 51.6± SD 11.5), their LDL ranged from 90.9 to 162.6 mg/dl (mean 130.2± SD 60.9), their fasting sugar ranged from 71.5 to 81.8 mg/dl (mean 78.9± SD 7.3), their sugar ranged from 90.0 to 100.0 mg/dl (mean 92.3± SD 8.6), their fasting insulin ranged from 7.1 to 81.5 IU/L (mean 43.6± SD 38.1), their insulin resistance ranged from 1.2 to 14.6 (mean 8.6± SD 7.8). Comparison between cases and controls as regards FoxO 1a & 3a genotypes: table (1), regarding FoxO gene 1a , the study demonstrated that among 50 obese patients, 46 had the homozygous wild genotype, 2 patients had the heterozygous genotype and 2 patients had the homozygous mutant genotype while the 50 control subjects had the homozygous wild genotype showing no polymorphism. This difference between 2 groups was statistically significant (P=0.04). On other hand the difference between the two groups regarding FoxO gene 3a was statistically insignificant (P=0.2).

Table 1: Comparison between cases and controls as regards FoxO gene 1a & 3a genotype

	Case (n=50)		Control (n=50)		P value
FoxO1a	**N**	**%**	**N**	**%**	
Wild	46	92.0	50	100.0	0.04 S
Hetero	2	4.0	0	0.0	
Homo	2	4.0	0	0.0	
FoxO3a					
Wild	48	96.0	50	100.0	0.2 NS
Hetero	1	2.0	0	0.0	
Homo	1	2.0	0	0.0	

NS= non=significant (P value > 0.05), S= significant

In respect to FOXO1 a; their was no statistically significant difference between the obese patients having wild genotype and those having non -wild type as regards the clinical data (age, sex, height, weight and BMI) [(P value > 0.05] ,while regarding the laboratory data only fasting insulin and insulin resistance showed statistically significant difference (P value > 0.05) between these two groups (table 2).

Table 2: Comparison between obese cases having FoxO 1a wild genotype and those having non-wild genotype as regards laboratory data

	Wild foxO 1a (n=46)	Non-Wild FoxO 1a (n=4)	P Value
Cholesterol	206.6 ± 70.5	199.3 ± 34.3	0.8 NS
TG	121.7 ± 40.4	115.0 ± 50.7	0.8 NS
HDL	51.4 ± 10.8	53.8 ± 20.4	0.7 NS
LDL	130.9 ± 61.9	122.5 ± 53.8	0.8 NS
FBS	78.7 ± 7.4	81.3 ± 6.3	0.5 NS
PPBS	92.6 ± 8.8	89.3 ± 6.5	0.5 NS
Fasting insulin	39.6 ± 37.1	89.0 ± 5.2	**<0.001 S**
Insulin resistance	7.8 ± 7.6	17.8 ± 1.5	**<0.001S**

ISSN: 0975-8585

The same with FoxO3 a as regards the clinical data where there was no statistically significant difference between the obese patients having wild genotype and those having non -wild type (P value > 0.05) in contrast to the laboratory data only fasting insulin and HDL showed statistically significant difference (P value > 0.05) between these two groups (table 3).

Table 3: Comparison between obese cases having FoxO 3a wild genotype and those having non-wild genotype as regards laboratory data

	Wild foxO 3a (n=48)	Non-Wild FoxO 3a (n=2)	P Value
Cholesterol	205.6 ± 68.4	216.5 ± 84.1	0.8 NS
TG	121.3 ± 41.3	119.0 ± 33.9	0.9 NS
HDL	50.8 ± 10.7	70.0 ± 19.8	**0.02 S**
LDL	130.6 ± 60.0	122.7 ± 110.7	0.9 NS
FBS	79.0 ± 7.4	76.0 ± 5.7	0.6 NS
PPBS	92.1 ± 8.6	97.5 ± 10.6	0.4 NS
Fasting insulin	41.8 ± 37.9	85.5 ± 6.4	**0.001 S**
Insulin resistance	8.3 ± 7.8	16.1 ± 2.4	0.2 NS

Upon studying the correlation (table 4) between FoxO 1a and FoxO 3a polymorphism with other parameters there was significant positive correlation between FoxO 1a polymorphism and both fasting insulin and insulin resistance (rho=0.376, P value= 0.007), (rho=0.372, P value= 0.008) respectively. Such significant correlations was not detected with FoxO3a and these two parameters. Similarly no significant correlation was detected between each of FoxO 1a & 3a polymorphism and other parameters (BMI, Cholesterol, TG, HDL, LDL, FBS, PPBS).

Table 4: Correlation between FoxO 1a and FoxO 3a polymorphism with other parameters

		FoxO1a	FoxO 3a
BMI	**Rho**	0.122	0.109
	P	0.4	0.453
Cholesterol	**Rho**	0.016	0.054
	P	0.913	0.711
TG	**Rho**	0.001	-0.01
	P	0.993	0.947
HDL	**Rho**	-0.037	0.221
	P	0.8	0.123
LDL	**Rho**	0.019	-0.029
	P	0.897	0.839
FBS	**Rho**	0.114	-0.057
	P	0.431	0.694
PPBS	**Rho**	-0.096	0.102
	P	0.509	0.479
Fasting insulin	**Rho**	0.376	0.222
	P	**0.007**	0.121
Insulin resistance	**Rho**	0.372	0.208
	P	**0.008**	0.148

NS= non=significant (P value > 0.05), S= significant (P < 0.05), rho= Spearman correlation coefficient

Positive correlation was detected between BMI and cholesterol level (P = 0.003), TG level (P = 0.008), LDL (P = 0.03), fasting insulin level (P = 0.021) as well as insulin resistance (P = 0.014), on other hand no statistically significant correlation was detected between BMI and other parameters (Table 5).

ISSN: 0975-8585

Table 5: Correlation of BMI with other parameters among cases:

		BMI
		Case
Cholesterol	**Rho**	0.41
	P	**0.003**
TG	**Rho**	0.372
	P	**0.008**
HDL	**Rho**	0.078
	P	0.589
LDL	**Rho**	0.412
	P	0.003
FBS	**Rho**	0.183
	P	0.203
PPBS	**Rho**	0.056
	P	0.699
Fasting insulin	**Rho**	0.327
	P	**0.021**
Insulin resistance	**Rho**	0.344
	P	**0.014**

NS= non=significant (P value > 0.05), S= significant (P < 0.05), rho= Spearman correlation coefficient,

DISCUSSION

Obesity, a major public health concern, is a multifactorial disease caused by both environmental and genetic factors. Obesity is associated with increased risk of chronic diseases and decreased health-related quality of life and overall life expectancy . It is also associated with substantially elevated healthcare cost. Although recent genome-wide association studies have identified many loci related to obesity or body mass index, the identified variants explain only a small proportion of the heritability of obesity. Better understanding of the interplay between genetic and environmental factors is the basis for developing effective personalized obesity prevention and management strategies ([14]

The forkhead transcription factor family is characterized by a winged-helix DNA binding motif and the forkhead domain . The mammalian forkhead transcription factors of the O class (FoxOs) have four members: FoxO1, FoxO3, FoxO4, and FoxO6. FoxO1 and FoxO3 are expressed in nearly all tissues. FoxO1 has been shown to enhance or diminish the clinical events such as diabetic complications, cardiomyopathy, and carcinogenesis either based on animal studies or projection from in vitro studies. FoxO1 functions as a negative transcriptional modulator of insulin sensing genes, which reduces insulin sensitivity, on other hand Foxa3 is the only Forkhead protein positively affecting adipocyte differentiation [15].

Previous research identified 16 polymorphic sites in the FoxO genes and examined their genetic association with BMI and the study revealed that one promoter SNP in the FoxO 3a gene was associated with increased BMI [16].

Moreover, through genome-wide linkage studies [17], chromosome 6q21 (where FoxO3a lies) has been identified as a region that may be liked to obesity.

Thus, we examined whether genetic variants of FoxO 1a and FoxO3a genes, members of superfamily Fox O genes, play role in the possible etiology of obesity as well as their correlation with increased risk of its complications regarding sugar , cholesterol and triglyceride metabolism, that is to say its correlation to increased risk of diabetes and dyslipidemia with consequent cardiovascular complications of obesity, aiming at developing novel approaches for the prevention and treatment of obesity.

Two metabolic problems have been recognized in our patients when compared to their corresponding healthy controls. These are dyslipidemia and insulin resistance.

Wang et al [15] stated that obesity is strongly associated with the cause of structural and functional changes of the artery. Oxidative stress and inflammation play a critical role in the development of obesity-induced cardiovascular disorders. This is compatible with our findings where dyslipidemia was manifested in the form of elevated cholesterol, triglyceride and low-density lipoprotein (LDL) levels in the obese patients compared to the healthy controls and this may increase their risk of having cardiovascular disease. This is also goes with [18], who stated that elevated triglyceride level and low HDL level increase the risk of CVD as strong as having high level of LDL- cholesterol.

Our results showed that BMI was positively correlated to cholesterol, triglyceride and LDL levels as well as fasting insulin. Contrary to [19] who stated that BMI showed no significant association with cholesterol and LDL.

Obese patients had insulin resistance with high fasting insulin levels. This may be due to the release of a high amount of non-esterified fatty acids (NEFA), glycerol hormones and proinflamatory cytokines from adipose tissue as stated by [20]. Resistance to insulin leads finally to type 2 diabetes taking in consideration that it has a direct link to changes in the lipid profile in obese patients as proved by [21] who stated that there is probable link between hyperlipidemia and insulin resistance giving importance to lipid lowering agents which may be a weapon that slow down the process of insulin resistance and type 2 diabetes.

Our study showed that the minor allele of FoxO1a existed in two patients as a homozygous form and another two patients as a heterozygous form, the remaining 46 patients had homozygous wild form of FoxO 1a. None of the controls had minor allele of FoxO1a. The difference between the two groups showed statistically significant difference (P = 0.04). Thus, our study showed that the presence of minor allele of FoxO1a increased susceptibility to obesity.

The result of this study showed that the minor allele of FoxO3a existed in one patient as a homozygous form and another one patient as a heterozygous form, , the remaining 48 patients had homozygous wild form of FoxO 3a. None of the controls had minor allele of FoxO3a. The difference between the two groups showed no statistically significant difference (P = 0.2).

This study proved that polymorphism in either gene FoxO 1a&3a existed only in obese patients but not in non-obese controls. So, there might be a relation between FoxO genes 1a& 3a polymorphisms and obesity. However further additional studies with large sample size to validate the relation of FoxO genes polymorphisms to obesity is essential.

The patients in our study were classified according to FoxO1a genotypes into those having homozygous wild type (46 patients) and those having minor allele in homozygous or heterozygous forms (non-wild, 4 patients). These 2 groups were compared as regards clinical data (age, sex, height, weight and BMI). No statistically significant difference was elicited between the two groups regarding the clinical data.

The link between FoxO1a and obesity was previously proved by Van Der and Coffer [22] who stated that forkhead transcriptional factor FoxO 1a function in adipose cells to couple insulin signaling to adipogenesis which involves switching preadipocyte from proliferation to terminal differentiation and so, polymorphism in FoxO 1a may lead to increase in BMI.

In addition, these 2 groups of patients (wild FoxO1a and non wild FoxO1a) were compared regarding the laboratory data (lipid profile, FBS, PPBS, fasting insulin and insulin resistance). No statistical significance between these two groups regarding their laboratory data except for fasting insulin (P=0.01) and insulin resistance(P=0.01) . This May support Graham and Daryl [23] who reported that there is hand in hand relationship between FoxO1a and insulin resistance.

As mentioned previously, BMI was directly correlated to fasting insulin as well as FoxO1a polymorphism and this can direct our attention to the possible link between FoxO1a polymorphism, obesity and insulin resistance.

This data may support the conclusion of Puigserver et al, [24] who said that FoxO1a promotes the transcription of genes that increase glucose production . So, this correlation indicated that if the individual has FoxO1a gene polymorphism, this patient will have strong chance to be obese, so must follow all strategies for obesity prevention and management including physical activity, diet control and behavior changes.

The patients in our study were classified according to FoxO3a genotypes into those having homozygous wild type (48 patients) and those having minor allele in homozygous or heterozygous forms (non-wild,2 patients). These 2 groups were compared as regards clinical data (age, sex, height, weight and BMI). No statistically significant difference was elicited between the two groups regarding the clinical data.

Jae- Ryong et al [16] showed a significant association with one promoter of single nucleotide polymorphism in the 5' flanking region of FoxO3a with BMI. However they did not find the effect of FoxO3a genetic polymorphisms to be powerful.

In addition these 2 groups of patients (wild FoxO3a and non- wild FoxO3a) were compared regarding the laboratory data (lipid profile, FBS, PPBS, fasting insulin and insulin resistance). No statistical significance between these two groups regarding their laboratory data except for HDL (P=0.02) and fasting insulin (P=0.01).

This may support Ushma et al [25] who stated that increase in insulin promotes the phosphorylation of nuclear and cytoplasmic FoxO3a and thus leads to increased cytoplasmic FoxO3a. The increase in insulin will lead to increase in FoxO3a.

Finally, Further recommendations include performing additional studies with large sample sizes to validate the effect of FoxO genes polymorphisms and obesity. Also, more studies are needed to investigate the different FoxO genes polymorphisms.

REFERENCES

[1] Jong Kilo Joo and Kyu Sup Lee.Pharmacotherapy for Obesity. (2014). J Menopausal Med. Dec; 20(3): 90–96

[2] Shukla AP, Buniak WI, et al. Treatment of obesity in 2015.Cardiopulm Rehabil Prev. Mar-Apr;35(2):81-92.

[3] Taylor R. S.: Use of Body Mass Index for Monitoring Growth and Obesity. (2010). Pediatr. & Child Health; vol. 15: 258.

[4] Carter LG, Qi NR, et al. Maternal exercise improves insulin sensitivity in mature rat offspring. (2013) Med Sci Sports Exerc ;45:832–840.

[5] Hu Y.H., Reilly K.H., Liang Y.J., et al. : Increase in Body Mass Index, Waist Circumference and Waist-to-Height Ratio is Associated with High Blood Pressure in Children and Adolescents in China. (2011) . J int Med Res; vol. 39: 23-32.

[6] Kopelman P.: Health risks associated with overweight and obesity. (2007). Obese Rev.; vol. 8: 13-17.

[7] DC Adler-Wailes, AT Alberobello, et al .Analysis of variants and mutations in the human winged helix FOXA3 gene and associations with metabolic traits. (2015). International Journal of Obesity , 1–5.

[8] Acilli D: The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic β cell growth. (2004). J Clin Invest; vol. 110: 1839– 1847.

[9] Nakae J., Biggs W.H., Kitamura T., et al.: Regulation of insulin action and pancreatic beta-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1. (2004). Nat. Genet.; 32: 245–53.

[10] Lorna M., Johanna Z. and Markus S.: Role of Central Insulin-Like Growth Factor-1 Receptor Signalling in Ageing and Endocrine Regulation. (2012). Basic and Clinical Endocrinology Up-to-Date; chapter 10.

[11] Kenneth M., Zhao Z. C., and Yan C. S.: OutFOXOing disease and disability: the therapeutic potential of targeting FoxO proteins. (2008). Trends Mol Med.; vol. 14: 219–227.

[12] Dansen T., Kops G., Denis S., et al : Regulation of sterol carrier protein gene expression by the forkhead transcription factor FOXO3a. (2004). J Lipid Res.; vol. 45: 81–88.

[13] Imae M., Fu Z., Yoshida A., et al: Nutritional and hormonal factors control the gene expression of FoxOs, the mammalian homologues of DAF-16. (2003). J Mol Endocrinol.; vol. 30: 253–262.

[14] Tao Huang and Frank B Hu. Gene-environment interactions and obesity: recent developments and

future directions, (2015). BMC Med Genomics. 8 (Suppl 1).
[15] Yu Wang, Yanmin Zhou, et al . FoxO Transcription Factors: Their Clinical Significance and Regulation. (2014). BioMed Research International. Volume (2014), Article ID 925350, 13 pages.
[16] Jae-Ryong K., Hong Soo J., Seong-Wook B., et al: Polymorphisms in FOXO gene family and association Analysis with BMI. (2006). J Korean Med Sci.; vol. 17: 75–80.
[17] Perusse, L., Rankinen, T., et al: The human obesity gene map: the 2004 update. (2005) . Obes., 13: 381-490.
[18] Gotto A.M Jr.: Cholesterol, inflammation and atherosclerotic cardiovascular disease: is it all LDL. (2011). Trans. Am. Clin. Climatol. Assoc.; vol. 122: 256–87.
[19] Shamai L., Lurix E., Shen M., et al.: Association of body mass index and lipid profiles: evaluation of a broad spectrum of body mass index patients including the morbidly obese. (2011). Obes Surg.; vol. 21:42-47.
[20] Mukherjee B., Chowdhury M. H., Laboni M., et al.: Obesity and Insulin Resistance: An Abridged Molecular Correlation. (2013). Lipid Insights; vol. 6: 1–11.
[21] Schaefer E.J., Gleason J.A. and Dansinger M.L.: Dietary fructose and glucose differentially affect lipid and glucose homeostasis. (2009). J. Nutr.; vol. 139: 1257S–1262S.
[22] Van D. V. and Coffer P. J.: The extending network of FOXO transcriptional target genes, (2011). Antioxidants and Redox Signaling; vol. 14: 579–592.
[23] Graham R.S. and Daryl P.S.: Computational modeling of the regulation of insulin signaling by oxidative stress. (2013). BMC Syst Biol. ; 7: 41.
[24] Puigserver P., Rhee J., Donovan J., et al: Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction. (2003). Nature; vol. 423: 550–555.
[25] Ushma R.J., Jiri Z., and Frederick M.S.: Insulin acts through FoxO 3a to activate transcription of Plasminogen Activator Inhibitor type 1. (2009). Mol Endocrinol. ; 23: 1487-1602.